ZooKeys 242: 1–16 (2012)
doi: 10.3897/zookeys.242.3996
www.zookeys.org

RESEARCH ARTICLE

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Compilation of morphological and molecular data, a necessity for taxonomy: The case of *Hormogaster abbatissae* sp. n. (Annelida, Clitellata, Hormogastridae)

Marta Novo[1,2,†], Rosa Fernández[1,3,‡], Daniel Fernández Marchán[1,§], Mónica Gutiérrez[3,|], Darío J. Díaz Cosín[1,¶]

1 *Departamento de Zoología y Antropología Física, Facultad de Biología, Universidad Complutense de Madrid, C/ José Antonio Nováis 2, 28040, Madrid, Spain* **2** *Cardiff School of Biosciences, Cardiff University, BIOSI 1, Museum Avenue, Cardiff CF10 3TL, UK* **3** *Museum of Comparative Zoology, Department of Organismic and Evolutionary Biology, Harvard University, 26 Oxford Street, Cambridge, MA 02138, USA*

† *urn:lsid:zoobank.org:author:79DA5419-91D5-4EAB-BC72-1E46F10C716A*
‡ *urn:lsid:zoobank.org:author:99618966-BB50-4A01-8FA0-7B1CC31686B6*
§ *urn:lsid:zoobank.org:author:CAB83B57-ABD1-40D9-B16A-654281D71D58*
| *urn:lsid:zoobank.org:author:E1A7E77A-9CD5-4D67-88A3-C7F65AD6A5BE*
¶ *urn:lsid:zoobank.org:author:38538B17-F127-4438-9DE2-F9D6C597D044*

Corresponding author: *Marta Novo* (mnovo@bio.ucm.es)

Academic editor: *R. Blakemore* | Received 18 September 2012 | Accepted 31 October 2012 | Published 15 November 2012

urn:lsid:zoobank.org:pub:C4149618-04B0-416B-9EA0-AE6AA6A95BF8

Citation: Novo M, Fernández R, Fernández Marchán D, Gutiérrez M, Díaz Cosín DJ (2012) Compilation of morphological and molecular data, a necessity for taxonomy: The case of *Hormogaster abbatissae* sp. n. (Annelida, Clitellata, Hormogastridae). ZooKeys 242: 1–16. doi: 10.3897/zookeys.242.3996

Abstract

Conflict among data sources can be frequent in evolutionary biology, especially in cases where one character set poses limitations to resolution. Earthworm taxonomy, for example, remains a challenge because of the limited number of morphological characters taxonomically valuable. An explanation to this may be morphological convergence due to adaptation to a homogeneous habitat, resulting in high degrees of homoplasy. This sometimes impedes clear morphological diagnosis of species. Combination of morphology with molecular techniques has recently aided taxonomy in many groups difficult to delimit morphologically. Here we apply an integrative approach by combining morphological and molecular data, including also some ecological features, to describe a new earthworm species in the family Hormogastridae, *Hormogaster abbatissae* **sp. n.**, collected in Sant Joan de les Abadesses (Girona, Spain). Its anatomical and morphological characters are discussed in relation to the most similar Hormogastridae species, which

Copyright *Marta Novo et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License 3.0 (CC-BY), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

are not the closest species in a phylogenetic analysis of molecular data. Species delimitation using the GMYC method and genetic divergences with the closest species are also considered. The information supplied by the morphological and molecular sources is contradictory, and thus we discuss issues with species delimitation in other similar situations. Decisions should be based on a profound knowledge of the morphology of the studied group but results from molecular analyses should also be considered.

Keywords
Species description, earthworm, morphological characters, molecular data, integrative taxonomy, homoplasy

Introduction

Traditional methods for identifying earthworm species and their phylogenetic relationships (i.e., the study of their morpho-anatomical features) have been limited by high levels of homoplasy. The structural simplicity of earthworms, the low degree of variability and the overlap of diagnostic characters among species, the absence of a fossil record and their adaptation to life in the soil, are the principal factors responsible for the difficulties in recognizing species. DNA sequence data has however facilitated the distinction of closely related species and may be the solution to understanding the true level of biodiversity within morphologically-difficult groups, such as earthworms.

Some degree of controversy has arisen on how to describe and delimit species, but discrete morphological features remain the most used criterion. Others are in favour of molecular-based descriptions (e.g., Cook et al. 2010) who justify species descriptions based solely on DNA sequences, even ignoring morphological data, but they also recognize that in cases with incomplete molecular databases—as for most taxa—, this alternative alone is not viable. Species descriptions including both morphological and DNA-based data are imperative for a more universal taxonomy. There are many authors in favor of this integrative taxonomy, consisting in a multidisciplinary approach including morphological, molecular, ecological and geographical data. This type of approach can include complex procedures therefore using multi-gene genetic distances, analyses such as General Mixed Yule-Coalescent (GMYC) models or Automatic Barcode Gap Discovery (ABGD) analyses and weighting of the established hypotheses with complementary data such as morphological, geographical or ecological (see Puillandre et al. 2012 and included references).

Hormogastridae includes middle to large-sized earthworms, currently comprising 27-29 species and subspecies that are exclusively distributed in the western Mediterranean (Díaz Cosín et al. 1989, Cobolli Sbordoni et al. 1992, Blakemore 2004, 2008), where they play a very important ecological role (Bouché 1972). The highest abundance of species seems to be located in the NE Iberian Peninsula, where more than a dozen species have been described.

The taxonomy of this group, as in other earthworm families, has been based until now solely on morphological features. The first species described are *Hormogaster redii* Rosa, 1887 and *Hormogaster pretiosa* Michaelsen, 1899. Subsequently, other species

were added to the group by different authors, including Cognetti (1914), Zicsi (1970), Bouché (1970), Álvarez (1971, 1977), Díaz Cosín et al. (1989), and Rota (1993) but most were described by Qiu and Bouché (1998), including eleven new species from Spain presenting very subtle morphological differences. The known species are grouped in four genera, *Hormogaster* Rosa, 1887 (22-24 species and subspecies), *Hemigastrodrilus* Bouché, 1970 (one or two subspecies), *Vignysa* Bouché, 1970 (two species) and *Xana* Díaz Cosín et al., 1989 (one species).

Omodeo (1956) provided the first revision of the family in 1956, and later on Omodeo and Rota (2008) presented additional considerations on their evolution in an article including different Mediterranean areas. Cobolli Sbordoni et al. (1992) provided the first phylogenetic hypothesis of the family using allozymes, but that seminal work lacks a comprehensive sampling in NE Spain, where most of the hormogastrid diversity concentrates (Qiu and Bouché 1998). More recently, Novo et al. (2009, 2010) used DNA sequence data from multiple markers to detect cryptic diversity within *Hormogaster elisae* in the central area of the Iberian Peninsula. These studies highlight, among other aspects, the morphological stasis present in this group, whose anatomy seems to have adapted to the dry soils of this region.

During a collecting trip for the phylogenetic study of Novo et al. (2011), 22 hormogastrid specimens were collected near Sant Joan de les Abadesses (Girona, Spain). The specimens were assigned to *Hormogaster*, but were thought to represent a new species here described as *Hormogaster abbatissae* sp. n. Its description, including its relationship to other the closely related hormogastrid species are the initial objectives of this paper. The description we provide is complemented with a molecular analysis of different genes in the closest species, GMYC species delimitation and ecological data. This study resembles the first example to describe a new earthworm species by combining all these different data sources (Blakemore and Kupriyanova 2010, see also Blakemore 2010, Blakemore et al. 2010 and for lumbricids Blakemore and Grygier 2011 and Blakemore 2012) and also other studies on different taxa (e.g. Chullasorn et al. 2011, Heethoff et al. 2011, Hart et al. 2012). However, this is the first work to do so for an hormogastrid. Contradictory results between morphological and molecular data are found, and whether a particular data set should be favored over the remaining sources is discussed.

We expect that this example, combining molecular and morphological data and including ecological features, goes beyond the specific interest of a new earthworm species description and could be applied to other groups with comparable taxonomic problems.

Material and methods

Specimens were collected by hand and fixed in the field in ca. 96% EtOH, with subsequent alcohol changes. Once in the laboratory, specimens were preserved at -20 °C.

The studied material includes 22 specimens (five mature specimens, four semimature specimens with tubercula pubertatis and/or clitellum draft and 13 immatures

or fragments) collected between Ripoll and Sant Joan de les Abadesses, road C26, km 210 in a little forest near the Ter river (42°13'30.0"N, 2°14'57.5"E). Mean annual temperature is 14.3 °C and mean annual precipitation is 724 mm, as indicated by the nearest weather station (in the airport of Girona, 55km away: http://www.aemet.es/es/serviciosclimaticos/datosclimatologicos/valoresclimatologicos?l=0367&k=cat)

Specimens have been deposited in the Oligochaete Cryo collection of the *Departamento de Zoología y Antropología Física, Universidad Complutense de Madrid* (DZAF, UCM), Spain.

Specimens of nearly all other hormogastrid species were examined for comparison (list of specimens in Novo et al. 2011). Morphological characters include those features traditionally used for hormogastrids and other earthworms. Only the distantly related species *H. lleidana* Qiu & Bouché, 1998 and *H. multilamella* Qiu & Bouché, 1998 were not examined, and thus their information was limited to the published descriptions (Qiu and Bouché 1998). All the specimens are deposited in the earthworm criocollection of Complutense University of Madrid (DZAF, UCM).

Molecular data generation follow Novo et al. (2011, 2012). Phylogenetic inference and GMYC analyses discussed here are based on data published in those papers. Nine molecular regions of specimens SAN1, 2, 3, 4, 7, 8, 9, 10 were included: mitochondrial regions of cytochrome *c* oxidase subunit I (COI), 16S rRNA and tRNA Leu, Ala, and Ser, two nuclear ribosomal genes (complete 18S rRNA and a fragment of 28S rRNA) and two nuclear protein-encoding genes (histones H3 and H4). GenBank accession numbers for the paragenetypes, following Chakrabarty (2010) for the mitochondrial markers, analyzed here are shown in Table 1.

We constructed networks with SplitsTree4 v.4.11.3 (Huson and Bryant 2006) for the mitochondrial genes (16S-tRNA, COI), including the phylogenetically closest species of *H. abbatissae* sp. n., in order to visualize in more detail the relationships and genetic distances among them. Default settings were used. We analysed 41 sequences of each gene including hormogastrids close to *H. abbatissae* sp. n. and *Hormogaster elisae* Álvarez, 1977 from Siguero and *Aporrectodea trapezoides* (Dugés 1828) as more distant outgroups (see Table 2). Uncorrected pairwise differences were calculated between these species with Arlequin 3.5 (Excoffier et al. 2005).

Table 1. Paragenetypes of *Hormogaster abbatissae* sp. n. with GenBank accession numbers. The holotype SAN 11 was not sequenced in order to preserve the specimen intact.

Paragenetype	COI	16S-tRNA
SAN1	JN209553	JN209358
SAN2	HQ621990	HQ621884
SAN3	JN209557	JN209360
SAN4	JN209555	JN209361
SAN7	JN209556	JN209362
SAN8	JN209559	JN209363
SAN9	JN209558	JN209364
SAN10	JN209554	JN209359

Table 2. Species represented in the network corresponding to the closest relatives of *H. abbatissae*, according to the phylogenetic study by Novo et al. (2011). More distantly related species appear in bold. GenBank accession numbers of the used sequences are shown for each gene.

Species	Locality	Region, Country	Coordinates	N	COI	16S
H. sylvestris	Montmajor	Barcelona, Spain	42°01'43.3"N, 001°42'43.7"E	2	JN209552, HQ621981	JN209286, HQ621874
H. pretiosa nigra	Quillan	Aude, France	42°52'48.8"N, 002°10'12.0"E	1	HQ621988	HQ621882
H. catalaunensis	El Brull	Barcelona, Spain	41°48'04.9"N, 002°20'51.6"E	1	HQ621973	HQ621866
H. gallica	Banyuls Sur Mer	Pyrénées-Orientales, France	42°28'08.0"N, 003°09'08.2"E	1	HQ621974	HQ621867
H. arenicola	Biosca	Lleida, Spain	41°51'04.6"N, 001°19'40.4"E	8	JN209493-JN209499, HQ621972	JN209208-JN209214, HQ621865
H. riojana	Alesanco	La Rioja, Spain	42°26'21.7"N, 002°50'18.4"W	10	JN209477-JN209485, HQ621970	JN209196-JN209204, HQ621862
H. ireguana	Torrecilla en Cameros	La Rioja, Spain	42°13'54.7"N, 002°37'35.2"W	8	JN209486-JN209492, HQ621994	JN209394-JN209400, HQ621888
H. elisae	Siguero	Madrid, Spain	41°11'06.1"N, 03°37'07.4"W	1	EF653894.1	GQ409710.1
A. trapezoides	San Román	Asturias, Spain	43°15'20.9"N, 005°05'10.3"W	1	JF313607	HQ621864

Results

Taxonomic results

Phylum Annelida Lamarck, 1802
Subphyllum Clitellata Michaelsen, 1919
Class Oligochaeta Grube, 1850
Order Haplotaxida Michaelsen, 1900
Family Hormogastridae Michaelsen, 1900
Genus *Hormogaster* Rosa, 1887

Type-species *Hormogaster redii* Rosa, 1887

***Hormogaster abbatissae* Novo & Díaz Cosín, sp. n.**
urn:lsid:zoobank.org:act:6A388AC5-A2E4-4A32-9BA4-F0F1C5684EBE
http://species-id.net/wiki/Hormogaster_abbatissae

Hormogaster abbatissae Novo, 2010: 249 (eprints.ucm.es/12304/1/T32615.pdf) and Novo and Díaz Cosín, in press: (http://www.ucm.es/info/zoo/invertebrados/PDF/

Novo%20et%20al%20%28en%20prensa%29%20When%20morphology%20and%20molecules%20clash.pdf) – nomina nuda superceded by current publication.

Material examined. *Holotype.* Adult (Catalog # SAN11 DZAF, UCM), 42°13'30.0"N, 2°14'57.5"E, from a small patch of forest near the Ter river, road C26, Km 210, between Ripoll and Sant Joan de les Abadesses, Girona (Spain), leg. M. Novo, D. Díaz Cosín, R. Fernández, December 2006.

Paratypes. 21 specimens (Catalog # SAN1-10, 12-22 DZAF, UCM), same collecting data as holotype.

Other material examined. 16 *Hormogaster* species and several subspecies included in the study by Novo et al. (2011).

Morphological description. *External morphology* (Figure 1). Length of the mature specimens: 103-130 mm. Maximum diameter (pre-clitellar, clitellar, post-clitellar): 8, 11, 9 mm. Number of segments: 239-270. Weight (fixed specimens): 3.45-4.98 g.

Colour: Anterior pink in live animals, with darker clitellum and grey-bluish posterior (Supplementary Figure S.1B). Specimens are grey-bluish when preserved in ethanol, with beige clitellum (Supplementary Figure S.1D).

Prostomium proepilobic 1/3. Segments 1 and 2 showing longitudinal lines. Chaetae closely paired, quite lateral, visible along the body as two faint blue lines; intersetal ratio at segment 50, *aa*: 50, *ab*: 1.5, *bc*: 9, *cd*: 1, *dd*: 52. Nephridial pores in a row, between chaetae *b* and *c*. Spermathecal pores at intersegments 8/9, 9/10 and 10/11, at the level of chaetae *cd*.

Male pores opening near the 15/16 as elongated fissures at the level of *ab*, showing heart-shaped porophores of variable developmental degree that can cover practically all width of the segment 15 and ½ of 16 in mature specimens. Female pores in 14 more or less at the same level as the male ones.

Clitellum saddle-shaped extending over 14,15–27. Tubercula pubertatis in (20) 21,22–26,27 appearing frequently as a continuous line in 21–27. Papillae with variable position, frequently situated at *ab* chaetae in segment 27, although more variable in other segments within the pre-clitellar and clitellar area.

Internal anatomy. Funnel-shaped and strongly thickened septa in 7/8, 8/9 and 9/10, also in 6/7 and 10/11, less thickened though. Last pair of hearts in 11. Three globular strongly muscular gizzards in 6, 7 and 8 of shining appearance. Not apparent Morren's glands, although in transverse sections of the oesophagus at segments 10 to 14 some thickened blood vessels can be detected, but never the lamellae typically showed by this glands.

Lack of well-differentiated posterior gizzard, although the esophagus is a bit dilated at 15–16, but its wall is not especially muscular and its lumen does not exhibit a reinforcement similar to that in the anterior gizzards. In segments 17–25, 26, the gut shows folds in the wall of every segment, forming what has been called a stomach in some earthworms. Typhlosole begins in 20, 21 and presents 15 lamellae, being the two lateral ones very small that therefore could be unnoticed. Number of lamellae gradually decreases, showing three from segment 80 to 140–150, and one until 160–170 where the typhlosole ends. Therefore the last 70 to 100 segments lack the typhlosole.

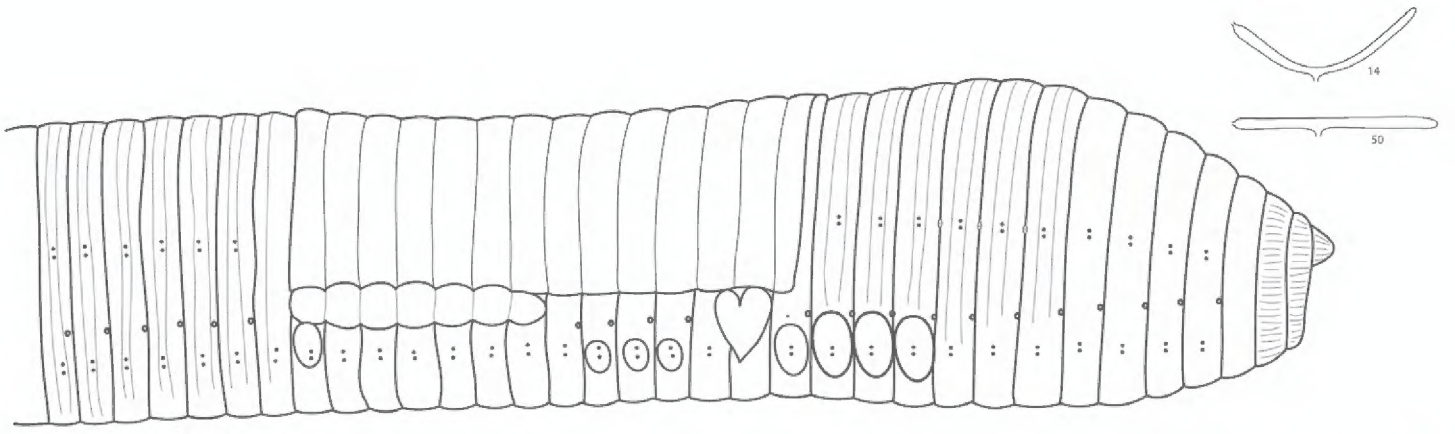

Figure 1. External morphology of *H. abbatissae*. An illustration of nephridial bladders in segments 14 and 50 is shown in the upper right corner.

Fraying testes and iridescent seminal funnels in 10 and 11. Two pairs of granular appearing seminal vesicles in 11 and 12 frequently showing black bodies. Ovaries and female funnels in 13; big ovarian receptacles in 14.

Three pairs of spermathecae in segments 8, 9 and 10 included into septa 8/9, 9/10 and 10/11 the ones in 8 being the smallest. Spermathecae with the appearance of flattened sacks, dish or flying saucer showing irregular borders inside the body wall under some of the muscular fascicles. They can be divided internally into interconnected lobes that in fact do not represent independent spermathecae but simple multicameral spermathecae that open to the exterior by a unique pore.

Anterior nephridial bladders V-shaped with widely open branches, being one of them shorter. They flatten towards the posterior section of the body, until the extent of showing appearance of an elongated sausage.

In some of the specimens, the sexual chaetae in 11 and 12 present well developed follicles that go into the body as a projection where various chaetae simultaneously appear.

Distribution. Known only from its type locality.

Habitat. Specimens were collected in a small forest patch dominated by *Populus alba*, *Acer pseudoplatanus* and *Rosa canina*, which develops in a slope at the edge of a meadow. The soil was covered with abundant leaf litter (Supplementary Figure S1. A), and it is characterized by 13.57% of coarse sand, 9.62% fine sand, 6.27% coarse silt, 32.37% fine silt, and 38.18% clay, constituting a clay loam soil, carbon (C): 4.48%, nitrogen (N): 1.32%, C/N: 3.39, pH: 7.09.

Etymology. The specific epithet derives from *abbatissa*, Latin for abbess, as the species is dedicated to the abbess Emma, the first Abbess head of the Monastery of Sant Joan de les Abadesses, founded in 885 AC by her father, the Count of Barcelona, Guifré el Pilós. The Monastery was run by nuns until the year 1,017 when the female community was expelled, presumably for disorderly conduct, and replaced by monks.

Molecular characters. Sequences from COI (8 individuals), 16S-tRNA (8 ind.), histone H3 (4 ind.), histone H4 (4 ind.), 28S rRNA (2 ind.) and 18S rRNA (1 ind.) were analysed with additional hormogastrid species. Phylogenetic analyses of the molecular data shows robust support for the monophyly of *Hormogaster abbatissae* sp. n., which is the sister species of *H. sylvestris* Qiu & Bouché, 1998 (Figure 2), described in

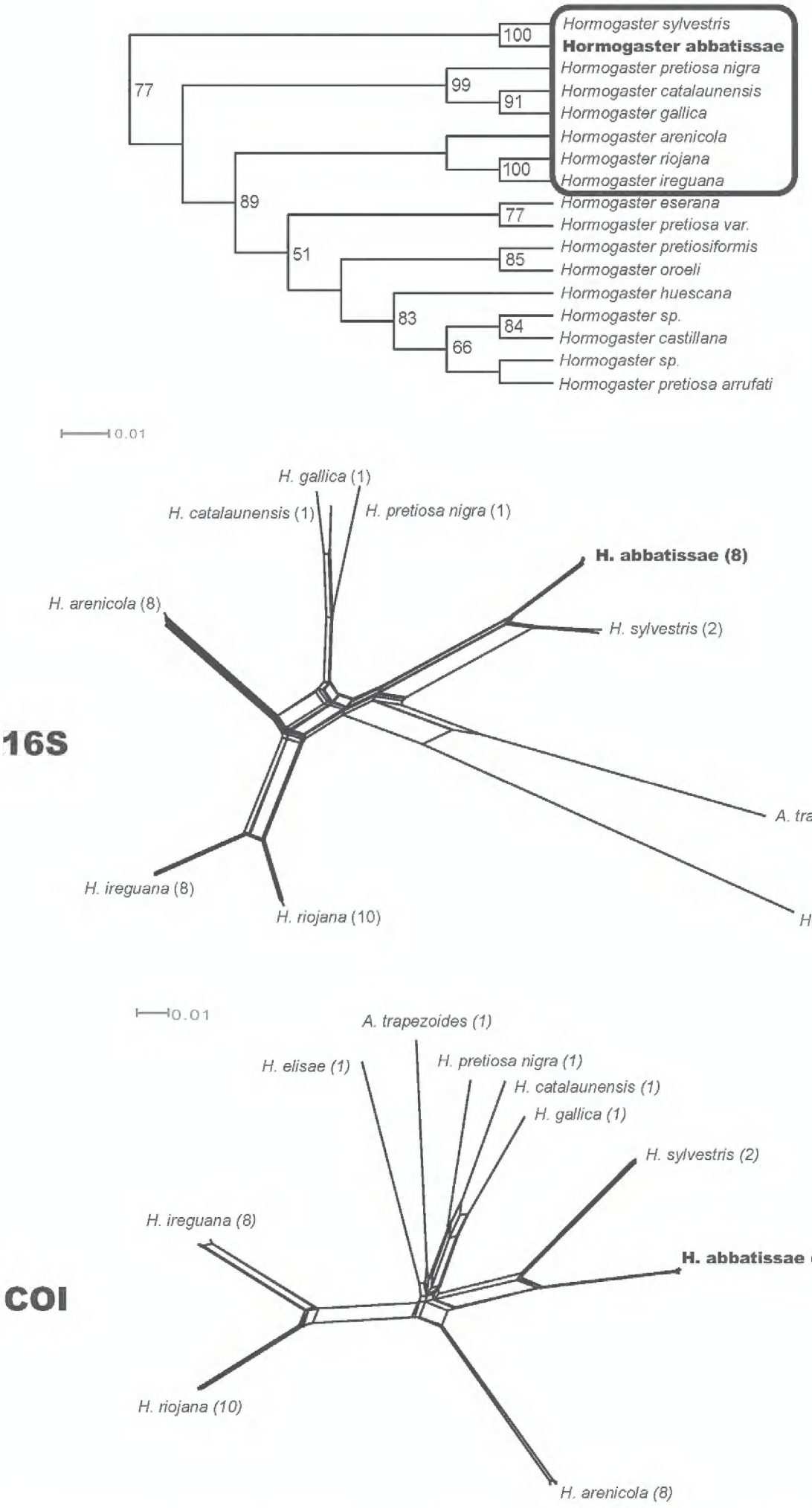

Figure 2. Top, part of the parsimony tree recovered by Novo et al. (2011), showing the clade where *H. abbatissae* was placed (in that work it is named *sp n.*). Bottom, network representation for 16S-tRNA and COI recovered by SplitsTree4 of the closest species (surrounded by a black square in the tree above) and *Hormogaster elisae* and *Aporrectodea trapezoides* as distant references. The number of specimens used is indicated in parenthesis.

the nearby locality of Montmajor (Barcelona, Spain). This clade forms the sister group to almost all other *Hormogaster* species from the NE Iberian Peninsula (see Novo et al. 2011 for details). This latter clade from the NE Iberian Peninsula splits into two groups, the first clade including *H. gallica* Rota, 1994 from Banyuls-sur-Mer (S of France), *H. catalaunensis* Qiu & Bouché, 1998 from El Brull (Barcelona, Spain) and *H. pretiosa nigra* Bouché, 1970 from Quillan (S of France). Its sister clade includes other *Hormogaster* species from the NE Iberian Peninsula, including *H. riojana* Qiu & Bouché, 1998 and related species (Figure 2).

Uncorrected pairwise distances for 16S-tRNA and COI are shown in Table 3 for the sister species *Hormogaster abbatissae* sp. n. and *H. sylvestris* and the morphologically-close *H. riojana* as well as its sister species *H. ireguana* Qiu & Bouché, 1998. *Hormogaster elisae* is included as a distant relative, even though it belongs to a possible new genus (see Novo et al. 2011).

The networks recovered by Splitstree4 for the COI and 16S genes including morphological and molecular closest species are shown in Figure 2.

GMYC analyses performed by Novo et al. (2012) identified *H. abbatissae, H. riojana* and *H. sylvestris* as different species.

Ecological characters. Soil characteristics in the localities where *H. abbatissae* sp. n., *H. riojana* and *H. sylvestris* occur are shown in Table 4. Differences in soil texture were detected: *H. sylvestris* and *H. riojana* inhabit Silt-loamy soils, whereas *H. abbatissae* sp. n. inhabits Clay-loamy soils. *Hormogaster abbatissae* sp. n. inhabits soils with a higher content in organic matter. Comparisons with the remaining species of the family were provided by Novo et al. (2012).

Table 3. Mean values of uncorrected pairwise differences in percentage obtained for 16S-tRNA (above the diagonal) and COI (below the diagonal, in bold) genes. Values of intraspecific differences are shown in the diagonal for the species that include more than one sequence type.

	H. abbatissae	*H. sylvestris*	*H. riojana*	*H. ireguana*	*H. elisae*
H. abbatissae	**0.10**/0.05	4.01	11.92	12.86	17.88
H. sylvestris	**11.71**	**0.46**/0.25	11.89	12.76	16.29
H. riojana	**17.80**	**17.36**	**0**/0.09	4.32	17.18
H. ireguana	**16.11**	**18.58**	**9.53**	**0.33**/0.03	17.72
H. elisae	**18.42**	**19.68**	**18.52**	**19.48**	-

Table 4. Soil characteristics in the sampling localities of *H. sylvestris* (Montmajor MAJ), *H. abbatissae* sp. n. (San Joan de les Abadesses, SAN) and *H. riojana* (Alesanco, ALE). CSand: coarse sand, FSand: fine sand, TSand: total sand, CSilt: coarse silt, FSilt: fine silt, Tsilt: total silt, Tex: textural class, SL: Silt loam, CL: Clay loam, C: percentage of carbon, N: percentage of nitrogen, C/N carbon/nitrogen relationship.

	CSand	**FSand**	**TSand**	**CSilt**	**FSilt**	**TSilt**	**Clay**	**Tex**	**C**	**N**	**C/N**	**pH**
MAJ	11.71	6.50	18.22	6.88	69.02	75.90	5.88	SL	2.98	0.83	3.6	7.39
SAN	13.57	9.62	23.18	6.27	32.37	38.64	38.18	CL	4.48	1.32	3.4	7.09
ALE	9.24	25.12	34.36	55.38	1.86	57.24	8.40	SL	1.63	0.30	5.33	7.33

Discussion

Most species within the genus *Hormogaster* are very similar morphologically, with the clitellum, tubercula pubertatis, spermathecae and typhlosole, in addition to size or colour, being the key morphological characters traditionally used for species diagnosis. Table 5 includes a comparison of the characters of *H. abbatissae* sp. n. with those of its closest congeners, showing a large degree of overlap in the distribution of these characters and their states. In this case we have a species that appears the closest morphologically, *H. riojana*, collected in Alesanco, a locality ca. 420 km from Sant Joan de les Abadesses, that can be distinguished by the body and clitellum colour, shape of the tubercula pubertatis and the number of spermathecae (although *H. riojana* specimens with three pairs of spermathecae have been reported by Novo 2010). This could lead to consider *H. abbatissae* sp. n. a variety of *H. riojana*. Nevertheless, as shown by the phylogenetic and phylogeographic analyses of molecular data (see Figure 2), *H. riojana* appears distantly related to *H. abbatissae* sp. n.

The sister group of *H. abbatissae* sp. n. is *H. sylvestris* (Figure 2), collected in Montmajor, 50 km away from Sant Joan de les Abadesses. These two species, closely related phylogenetically and biogeographically, are easily distinguished by their tubercula pubertatis (generally starting in more anterior segments and finer in *H. abbatissae* sp. n), clitellum (shorter and saddle shaped in *H. abbatissae* sp. n. and annular in *H. sylvestris*), spermathecae (three pairs in *H. abbatissae* sp. n. and two pairs in *H. sylvestris*) and typhlosole (15 lamellae in *H. abbatissae* sp n. and 13 in *H. sylvestris*). To these characters we can add other more variable characters such as colour, length, weight and number of segments (*H. sylvestris* is longer, heavier and with a higher number of segments). Of all these characters, the presence of three pairs of spermathecae in *H. abbatissae* sp. n. is the most conspicuous trait. It is therefore the combination of the morphological information and the phylogenetic position of the species, as derived from the molecular data, which aids in the global taxonomy of the group and serves to assess the degree of homoplasy in characters thought to be of taxonomic importance.

Some characters, such as the presence of Morren's glands or the existence of a posterior gizzard, can be difficult to observe and of subjective interpretation. Morren's glands seem to be absent because although an enrichment of blood vessels is detected in the oesophageal wall of some segments 10 – 14, the lamellae that define this organ were never observed. Likewise, the presence of a posterior gizzard is difficult to determine, as the gut thickens in segments 15 – 19 in the members of some species. However, in *H. abbatissae* sp. n. there is neither strong musculature, nor the thickening and hard covering of the lumen as observed in the gizzards of earthworms.

Regarding the molecular characters, Novo et al. (2009, 2010) proposed the presence of five cryptic species within the *H. elisae* complex, which resulted to be separated by genetic divergences between 9.41 – 18.31% for cytochrome *c* oxidase subunit I (Kimura 2-parameter distances, whose values are slightly higher than the uncorrected distances, used here). Also Hebert et al. (2003) reported comparable divergences for the same marker between 11.3%, for congeneric species of various animal groups and

Table 5. Comparison of the morphological characters of *H. abbatissae* sp. n. with those in the morphologically closest species. N. segments: number of segments. N. typhlosole lamellae: number of typhlosole lamellae. Size, weight and number of segments are for adult specimens. For complete information of the rest of the species within Hormogastridae, see Qiu and Bouche (1998).

	H. abbatissae	***H. gallica***	***H. riojana***	***H. sylvestris***	***H. ireguana***
Colour	Grey-bluish	Dark brownish	Dark brownish	Colourless	Brownish-grey
Clitellum	14, 15–27 (28) Saddle shaped, beige	(13) 14–28 (29,30)* Saddle shaped	13,14, 17–27,28 Saddle shaped, dark	15–28 Annular	13–27 Annular
Tubercula pubertatis	(20) 21,22–26,27 Fine band	(22, 23) 24 – 27 Fine and short band	(20)21–27 Fine band	22–27 Wide band	19–26 Linear band
Intersetal ratio	50:1.5:9:1:52	69:1.3:8.8:1:66	55:1:13:1:65	50:2:10:1:50	120:1:20:1:100
Length	103–130	165–190	125–185	180–220	100
N. segments	239–270	250–433	243–278	350–420	223
Weight (g)	3.45–4.98	9.2–17		13.6–15.3	
Spermathecae (pores) Appearance	8,9,10 (8/9,9/10,10/11) Simple, Multicameral	9, 10 (9/10,10/11) Multiple, sessile, in a ring	9, 10 (9/10,10/11) Simple, Multicameral	9, 10 (9/10,10/11) Simple, Multicameral	8, 9, 10 (8/9,9/10,10/11) Simple
N. typhlosole lamellae	15 (2 very small)	13	15	13	19
Morren gland	Absent	Absent	Absent	Absent	Absent
Posterior gizzard	15? 16 17? Very weak	14–16? Weak	15–16 Weak	16 Weak	14–15 Weak
Other characters		Carinated anterior segments			

15.7% between annelid species. It is evident, though that strict phenetic distances cannot be used for delimiting taxonomic boundaries, as other studies have shown that the same marker may have within species divergences much larger than the ones proposed by Hebert et al. (2003) (e.g., Barber et al. 2006, Boyer et al. 2007). This has been debated for earthworms by Chang and James (2011), who proposed that differences among species are indeed clade-specific, but they propose the existence of a consensus for COI (Kimura-corrected) distances: values under 9% normally indicate the same species, while values above 15% most probably indicate different species and values between 9-15% can be ambiguous. The species pairs *Hormogaster abbatissae* sp. n. and *H. sylvestris* as well as *H. riojana* and *H. ireguana* present COI uncorrected divergences within this ambiguity range (11.71% and 9.53%, respectively). The latest species were described by Qiu and Bouché (1998) based on morphology. Therefore, it seems that in this case distances need to be treated cautiously, thus reinforcing the critiques of their use for species delimitation (DeSalle et al. 2005, Hickerson et al. 2006, Whitworth et al. 2006). However, distances seem to be conservative in hormogastrid's case and our data suggest that divergence below the level proposed by Chang and James (2011) may correspond to different species. Anyway it is clear the necessity of morphological data to verify the status of two lineages that present a divergence value

within this range. Moreover, in the present case, these species are known to appear only in their type locality and therefore barcoding gap (ABGD) cannot be calculated with accuracy. Species delimitation with GMYC has been recently implemented in earthworms by Fernández et al. (2012) and particularly in hormogastrids by Novo et al. (2012). In both cases an overestimation of the species number, when compared with morphology, was detected. The marked genetic structure and scarce dispersion capacity of the studied earthworms could be the cause for this overestimation, being these factors particularly evident in *H. elisae*'s case, with various cryptic species. A GMYC analysis shows *H. abbatissae* sp. n. as a different entity from *H. sylvestris*. Whether the GMYC method is overestimating in this particular case is unknown but *H. abbatissae* sp. n., *H. riojana* and *H. sylvestris* are well-separated when combining morphology, phylogenetic analyses and network information.

After examining its morphology, phylogenetic placement and additional data such as GMYC and soil characteristics, it is evident that *H. abbatissae* sp. n. constitutes a new hormogastrid taxon not phylogenetically related to those species that show closest morphological similarities. Morphological and molecular data supply different signals thus clashing in the case of *H. abbatissae* sp. n. The question arising in this case is what should taxonomists do when different data sources provide conflict? The answer to this question is not straightforward. On the one hand, these animals can present a morphological stasis, as shown in *H. elisae* (Novo et al. 2009, 2010). On the other hand, molecular techniques rely on limited information, in this case based on a group of specific genes and depend on specific algorithms. This decision should thus be based on a profound knowledge of the morphological variability and peculiarities of the studied group, and an understanding of the strengths and weakness of the applied molecular analyses (used genes, sampling scheme, algorithms, etc.) that could lead to different decisions depending on the study case.

In this particular case, phylogeny is robust because it is based on a great amount of data, combining mitochondrial and nuclear genes (COI, 16S-tRNA, H3, H4, 28S, 18S) with different phylogenetic signal and including individuals representing most of the species in the family. Also we know that living conditions in the soil induce cryptic speciation processes in earthworms (King et al. 2008, Novo et al. 2009, 2010, James et al. 2010, Buckley et al. 2011, Dupont et al. 2011 – but see rebuttals of these "cryptic" genetic cladograms by Blakemore et al. 2010 and Blakemore 2010, 2011) and in many occasions the most important morphological characters used for earthworm species delimitations overlap showing a poor discrimination capacity (Fernández et al. 2012). Therefore, morphological characters should be applied cautiously by earthworm taxonomists in case of conflict with other data source.

Regarding ecological factors, some important differences are detected for texture and organic matter among soils of *H. abbatissae* sp. n., *H. sylvestris* and *H. riojana*. However, it should be considered that a single locality is known per species and that the discovery of other populations may show a higher ecological range.

In summary, this study evidences the need of complementing the morphological data with molecular characters data in taxonomy, especially in groups with limited

morphological characters and rampant convergence in their functional morphology, perhaps due to strong selective pressure due to habitat restriction. This study also proves that in case of rather small genetic divergence (within the range of uncertainty), morphology can be also helpful to conclude complementing molecular sources. We propose to establish the new species *Hormogaster abbatissae* sp. n. Given the existence of species closely-related phylogenetically (*H. sylvestris*) and an unrelated but morphologically similar species (*H. riojana*), a more exhaustive sampling effort in NE Spain could provide new diversity to help evaluate this situation. As indicated by Sites and Crandall (1997), species descriptions are not facts, but hypothesis established when certain criteria available in a specific moment are fulfilled and they can be accepted or rejected when new data are available.

Acknowledgements

We are indebted to Fernando Pardos for the drawing of Figure 1 and to Gonzalo Giribet for his valuable comments on the manuscript. Subject Editor Rob Blakemore and three anonymous reviewers helped to improve a previous version. M.N. was supported by a Grant from the *Fundación Caja Madrid* and a Postdoctoral Fellowship from Spanish Government. R.F. was supported by a research contract from Spanish Government and a Postdoctoral Fellowship from *Fundación Ramón Areces*. This research was funded by project CGL2010-16032 from the Spanish Government.

References

Álvarez J (1971) Oligoquetos terrícolas ibéricos. Megascolécidos y Glososcolécidos. Boletin de la Real Sociedad Española de Historia Natural (Sección Biológica) 69: 97–114.

Álvarez J (1977) El género *Hormogaster* en España. Publicaciones del Centro Pirenaico de Biología Experimental 9: 27–35.

Barber P, Boyce SL (2006) Estimating diversity of Indo-Pacific coral reef stomatopods through DNA barcoding of stomatopod larvae. Proceedings of the Royal Society: B 273: 2053–2061. doi: 10.1098/rspb.2006.3540

Blakemore RJ (2004) A provisional list of valid names of Lumbricoidea (Oligochaeta) after Easton, 1983. In: Moreno AG, Borges S (Eds) "Avances en taxonomia de lombrices de tierra/ Advances in earthworm taxonomy (Annelida: Oligochaeta)". Editorial Complutense, Universidad Complutense, Madrid, Spain, 75–120. doi: 10.3897/zookeys.160.2354

Blakemore RJ (2008) An updated list of valid, invalid and synonymous names of Criodriloidea and Lumbricoidea (Annelida: Oligochaeta: Criodrilidae, Sparganophilidae, Ailoscolecidae, Hormogastridae, Lumbricidae, Lutodrilidae). A Series of Searchable Texts on Earthworm Biodiversity, Ecology and Systematics from Various Regions of the World - Supplemental. CD publication under ICZN (1999: Art. 8) also presented online http://www.annelida.net/earthworm/ [accessed Oct., 2012]

Blakemore RJ (2010) Unraveling some Kinki worms (Annelida: Oligochaeta: Megadrili: Megascolecidae) Part II. Opuscula Zoologica 42(2): 191–206.

Blakemore RJ (2011) Further records on non-cryptic New Zealand earthworms. Zookeys 160: 23–46.

Blakemore RJ (2012) On Schmarda's lost worm and some newly found New Zealand species. Journal of Species Research 1(2): 105–132.

Blakemore RJ, Grygier MJ (2011) Unraveling some Kinki worms (Annelida:Oligochaeta:Meg adrili:Lumbricidae) Part III. Journal of Soil Organisms 83(2): 231–244.

Blakemore RJ, Kupriyanova EK (2010) Unraveling some Kinki worms (Annelida: Oligochaeta: Megadrili: Moniligastridae) Part I. Opuscula Zoologica 40: 3–18.

Blakemore RJ, Kupriyanova E, Grygier MJ (2010) Neotypification of *Drawida hattamimizu* Hatai, 1930 (Oligochaeta: Megadrili: Moniligastridae) and the first COI sequence from an earthworm type. ZooKeys 41: 1–29.

Bouché MB (1970) Remarques sur quelques Lumbricina de France et consequences de la decouverte des noveaux taxons Vignysinae (Subfam. nov) et Diporodrilidae (Fam. nov.). Pedobiologia 10: 246–256.

Bouché MB (1972) Lombriciens de France Écologie et Systématique. Annales de Zoologie Écologie animale. Numéro hors série, Institut National de la Reserche Agronomique Paris, 671 pp.

Boyer SL, Baker JM, Giribet G (2007) Deep genetic divergences in *Aoraki denticulata* (Arachnida, Opiliones, Cyphophthalmi): a widespread 'mite harvestman' defies DNA taxonomy. Molecular Ecology 16: 4999–5016. doi: 10.1111/j.1365-294X.2007.03555.x

Buckley TR, James S, Allwood J, Bartlam S, Howitt R, Prada D (2011) Phylogenetic analysis of New Zealand earthworms (Oligochaeta: Megascolecidae) reveals ancient clades and cryptic taxonomic Diversity. Molecular Phylogenetics and Evolution 58: 85–96. doi: 10.1016/j.ympev.2010.09.024

Chakrabarty P (2010) Genetypes: a concept to help integrate molecular phylogenetics and taxonomy. Zootaxa 2632: 67–68.

Chang CH, James S (2011) A critique of earthworm molecular phylogenetics. Pedobiologia 54(Supplement S3–S9).

Chullasorn S, Dahms HU, Lee KW, Ki JS, Schizas N,Kangtia P, Park HG, Lee JS (2011) Description of *Tisbe alaskensis* sp. nov. (Crustacea: Copepoda) combining structural and molecular traits. Zoological Studies 50(1): 103–117.

Cobolli Sbordoni M, De Matthaeis E, Alonzi A, Mattoccia M, Omodeo P., Rota, E (1992) Speciation, genetic divergence and palaeogeography in the Hormogastridae. Soil Biology and Biochemistry 24: 1213–1221. doi: 10.1016/0038-0717(92)90096-G

Cognetti de Martiis L (1914) Escursioni Zoologiche del Dr. Enrico Festa nei monti della Vallata del Sangro (Abruzzo). Nota sugli Oligocheti degli Abruzzi. Bolletino dei Musei di Zoologia ed Anatomia Comparata della R. Universitá di Torino 689 (XXIX).

Cook LG, Edwards RD, Crisp MD, Hardy NB (2010) Need morphology always be required for new species descriptions? Invertebrate Systematics 24: 322–326. doi: 10.1071/IS10011

DeSalle R, Egan MG, Siddall M (2005) The unholy trinity: Taxonomy, species delimitation and DNA barcoding. Philosophical Transactions of the Royal Society (Biological Sciences Series) 360: 1975–1980. doi: 10.1098/rstb.2005.1722

Díaz Cosín DJ, Briones MJI, Trigo D (1989) Descripción de una nueva especie de lombriz de tierra, *Xana omodeoi* (Hormogastridae, Oligochaeta) y sus implicaciones en la división de los Hormogastridae. Revue d'Écologie et Biologie du Sol 26: 225–231.

Dupont L, Lazrek F, Porco D, King RA, Rougerie R, Symondson WOC, Livet A, Richard B, Decaens T, Butt KR, Mathieu J (2011) New insight into the genetic structure of the *Allolobophora chlorotica* aggregate in Europe using microsatellite and mitochondrial data. Pedobiologia 54: 217–224. doi: 10.1016/j.pedobi.2011.03.004

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1: 47–50.

Fernández R, Almodóvar A, Novo M, Simancas B, Díaz Cosín DJ (2012) Adding complexity to the complex: New insights into the phylogeny, diversification and origin of parthenogenesis in the *Aporrectodea caliginosa* species complex (Oligochaeta, Lumbricidae). Molecular Phyogenetics and Evolution 64: 368–379. doi: 10.1016/j.ympev.2012.04.011

Hart JA, Detwiler KM, Gilbert CC, Burrell AS, Fuller JL, Emetshu M, Hart TB, Vosper A, Sargis EJ, Tosi AJ (2012) Lesula: A New Species of *Cercopithecus* Monkey Endemic to the Democratic Republic of Congo and Implications for Conservation of Congo's Central Basin. Plos One 7: e44271. doi: 10.1371/journal.pone.0044271

Hebert PDN, Ratnasingham S, deWaard JR (2003) Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of the Royal Society of London, Series B (Suppl.) 270: S96-S99. doi: 10.1098/rsbl.2003.0025

Heethoff M, Laumann M, Weigmann G, Raspotnig G (2011) Integrative taxonomy: Combining morphological, molecular and chemical data for species delineation in the parthenogenetic *Trhypochthonius tectorum* complex (Acari, Oribatida, Trhypochthoniidae). Frontiers in Zoology 8:2. doi: 10.1186/1742-9994-8-2

Hickerson MJ, Meyer CP, Moritz C (2006) DNA barcoding will often fail to discover new animal species over broad parameter space. Systematic Biology 55: 729–739. doi: 10.1080/10635150600969898

Huson DH, Bryant D (2006) Application of Phylogenetic Networks in Evolutionary Studies. Molecular Biology and Evolution 23: 254–267. doi: 10.1093/molbev/msj030

James S, Porco D, Decäens T, Richard B, Rougerie R, Erséus C (2010) Barcoding reveals cryptic diversity in *Lumbricus terrestris* L., 1758 (Clitellata): Resurrection of *L. herculeus* (Savigny, 1826). PLos One 5: e15629. doi: 10.1371/journal.pone.0015629

King RA, Tibble AL, Symondson WOC (2008) Opening a can of worms: unprecedented sympatric speciation within British lumbricid earthworms. Molecular Ecology 17: 4684–4698. doi: 10.1111/j.1365-294X.2008.03931.x

Michaelsen W (1899) Revision der Kinberg'schen Oligochaeten-Type, Öfversigt Akademiens Förhandlingar, Stockholm 56: 413–448.

Novo M (2010) Algunos aspectos de la Biologia Evolutiva de la Familia Hormogastridae Michaelsen, 1900: Filogenia, Filogeografia y Seleccion Sexual. PhD Thesis, Univesidad Complutense de Madrid, Spain, 341 pp.

Novo M, Almodóvar A, Díaz Cosín DJ (2009) High genetic divergence of hormogastrid earthworms (Annelida, Oligochaeta) in the central Iberian Peninsula: evolutionary and demographic implications. Zoologica Scripta 38: 537–552. doi: 10.1111/j.1463-6409.2009.00389.x

Novo M, Almodóvar A, Fernández R, Trigo D, Díaz Cosín DJ (2010) Cryptic speciation of hormogastrid earthworms revealed by mitochondrial and nuclear data. Molecular Phylogenetics and Evolution 56: 507–512. doi: 10.1016/j.ympev.2010.04.010

Novo M, Almodóvar A, Fernández R, Giribet G, Díaz Cosín DJ (2011) Understanding the biogeography of a group of earthworms in the Mediterranean basin—the phylogenetic puzzle of Hormogastridae (Clitellata: Oligochaeta). Molecular Phylogenetics and Evolution 61: 125–135. doi: 10.1016/j.ympev.2011.05.018

Novo M, Almodóvar A, Fernández R, Trigo D, Díaz Cosín DJ, Giribet G (2012) Appearances can be deceptive: different diversification patterns within a group of Mediterranean earthworms (Oligochaeta, Hormogastridae). Molecular Ecology 21: 3776–3793. doi: 10.1111/j.1365-294X.2012.05648.x

Omodeo P (1956) Sistematica e distribuzione geografica degli Hormogastrinae (Oligocheti). Archivio botanico e biogeografico italiano 32: 159–177.

Omodeo P, Rota E (2008) Earthworm diversity and land evolution in three mediterranean districts. Proceedings of the California Academy of Sciences, Fourth Series 59 (I, N. 5): 65–83.

Puillandre N, Modica MV, Zhang Y, Sirovich L, Boisselier MC, Cruaud CM, Holford M, Samadi S (2012) Large-scale species delimitation method for hyperdiverse groups. Molecular Ecology 21: 2671–2691. doi: 10.1111/j.1365-294X.2012.05559.x

Qiu JP, Bouché MB (1998) Contribution to the taxonomy of Hormogastridae (Annelida: Oligochaeta) with description of new species from Spain. Documents Pedozoologiques et Integrologiques 4: 164–177.

Rosa D (1887) *Hormogaster redii* n. g., n. sp. Bollettino dei Musei di Zoologia ed Anatomia comparata della Universitá di Torino 32 (II).

Rota E (1993) Contribution to the taxonomy of the Hormogastridae (Annelida: Oligochaeta) with description of two new species from southern France. Journal of Natural History 28: 27–36. doi: 10.1080/00222939400770031

Sites JW, Crandall KA (1997) Testing species boundaries in biodiversity studies. Conservation Biology 11: 1289–1297. doi: 10.1046/j.1523-1739.1997.96254.x

Whitworth TL, Dawson RD, Magalon H, Baudry E (2007) DNA barcoding cannot reliably identify species of the blowfly genus *Protocalliphora* (Diptera: Calliphoridae). Proceedings of the Royal Society B (Biological Sciences) 274: 1731–1739. doi: 10.1098/rspb.2007.0062

Zicsi A (1970) Neue Regenwürmer (Oligochaeta: Hormogastridae, Lumbricidae) aus Spanien. Opuscula Zoologica (Budapest) X: 371–373.

Appendix

Supplementary figure. (doi: 10.3897/zookeys.242.3996.app) File format: Adobe PDF file (pdf).

Explanation note: Sampling area of *H. abbatissae* (A), alive specimen (B), fixed specimens (D) and their spermathecae from one side of the body (C) numbered from anterior to posterior.

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Citation: Novo M, Fernández R, Fernández Marchán D, Gutiérrez M, Díaz Cosín DJ (2012) Compilation of morphological and molecular data, a necessity for taxonomy: The case of *Hormogaster abbatissae* sp. n. (Annelida, Clitellata, Hormogastridae). ZooKeys 242: 1–16. doi: 10.3897/zookeys.242.3996.app